tase, 40%; NADH–ferricyanide oxidoreductase, 15%; NADPH–ferricyanide oxidoreductase, 27%. Acid phosphatase was 27% sedimentable.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970). *Biochem. J.* **116**, 40 P.

Changes in Subcellular Distribution of Enzymes during Respiratory Adaptation of *Saccharomyces carlsbergensis*

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Anaerobically grown cultures of *Saccharomyces carlsbergensis* [containing glucose (10%, w/v), ergosterol and Tween 80] were allowed to adapt in the growth medium by sparging the fermenters with air. Adaptation was arrested by the addition of cycloheximide and chloramphenicol. Homogenates prepared anaerobically from sphaeroplasts of yeast cells after respiratory adaptation were analysed by isopycnic zonal centrifugation as described by Cartledge, Howells & Lloyd (1970a).

After adaptation for 10 min, both cytochrome *c* oxidase and catalase were detectable (4.6 and 4.9 nmol/min per mg of protein respectively). Cytochrome *c* oxidase was found mainly (66%) at ρ 1.225, whereas catalase was virtually all non-sedimentable (95%) although some enzyme was detected at ρ 1.19. The distribution of NADH- and NADPH-cytochrome *c* oxidoreductase and NADH- and NADPH-ferricyanide oxidoreductase was similar to that found in anaerobically grown non-adapted cells (Cartledge, Howells & Lloyd, 1970b). Thus the distribution of newly synthesized cytochrome *c* oxidase was similar to, but not identical with, that of those enzymes of particles of population B found in anaerobically grown cells.

After 30 min adaptation the specific activities of cytochrome *c* oxidase and catalase had increased (26.3 and 8.6 nmol/min per mg of protein respectively) and a marked change in distribution of cytochrome *c* oxidase, adenosine triphosphatase and NADH-cytochrome *c* oxidoreductase had occurred. Although NADH-cytochrome *c* oxidoreductase and cytochrome *c* oxidase were still detectable (6.3% and 8.3% of the total respectively) at ρ 1.21–1.22, both enzymes also showed a broad distribution up to ρ 1.15. Although a portion of both enzymes corresponded with the peak of soluble protein the heterogeneous density distribution suggested that both enzymes (and also adenosine triphosphatase) were associated with either slowly sedimenting particles or particles of $\rho < 1.15$.

After adaptation for 1 h the specific activities of cytochrome *c* oxidase and catalase were 37.8 and

8.9 nmol/min per mg of protein respectively; succinate-cytochrome *c* oxidoreductase was also now detectable (6.2 nmol/min per mg of protein). Again most of the cytochrome *c* oxidase (83.4%) and NADPH-cytochrome *c* oxidoreductase (92%) showed $\rho < 1.15$; three other peaks of activity of cytochrome *c* oxidase were present at ρ 1.13, 1.18 and 1.225. Succinate-cytochrome *c* oxidoreductase had a broad distribution in the soluble region and at ρ 1.18. The sedimentable oligomycin-sensitive adenosine triphosphatase showed a broad distribution between ρ 1.12 and ρ 1.20 (median ρ 1.16).

'Soluble' adenosine triphosphatase (50% of the total) was not oligomycin-sensitive.

Distribution of acid phosphatase did not alter during adaptation, although the specific activity of whole homogenates showed a threefold increase over the anaerobic level of activity after 1 h adaptation.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970a). *Biochem. J.* **116**, 24 P.

Cartledge, T. G., Howells, L. & Lloyd, D. (1970b). *Biochem. J.* **116**, 40 P.

The Functional Characterization of Mitochondria from Rat Liver and Krebs II Ascites-Tumour-Cell Mitochondria

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Mitochondria synthesize proteins both in intact cells and after isolation (Work, Coote & Ashwell, 1968). Reports have appeared that mitochondria from animal cells contain a 55S ribosome-like particle (O'Brien & Kalf, 1967a,b). We find that washed sterile mitochondria from liver or Krebs cells can be pulse-labelled with [^{14}C]leucine or [^{35}S]methionine. After short pulses (up to 5 min) most of the radioactivity is found attached to a particle sedimenting with *Escherichia coli* ribosome fraction in sucrose gradients containing 10 mM-Mg $^{2+}$. After longer periods of incubation or after a 'chase' of unlabelled leucine there is transfer of radioactivity to membrane proteins.

The incorporation of [^{14}C]leucine into the radioactive peak is not inhibited by the presence of cycloheximide (100 $\mu\text{g/ml}$) or ribonuclease (20 $\mu\text{g/ml}$) during the pulse, but the presence of D-threo-chloramphenicol (100 $\mu\text{g/ml}$) will cause 80% inhibition of incorporation. The radioactivity attached to the ribosomal particles is presumed to be in the form of incomplete peptide chains, since exposure of mitochondria to puromycin for 30 s or longer under conditions favouring protein synthesis results in complete removal of radioactivity from the peak; moreover this effect of puromycin is partially blocked by chloramphenicol. The